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ABERDEEN PROVING GROUND, MARYLAND 21010-5425

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EFFECTS OF CHRONIC PYRIDOSTIGMINE ADMINISTRATION ON SUSTAINED WORK OUTPUT IN MONKEYS

DAVID M. PENETAR

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Two pairs of cynomolgus monkeys were tested with repeated chronic pyridostigmine administration to assess this drug's effects on an operant schedule requiring large amounts of sustained work. One monkey of each pair earned reinforcement on a progressive ratio lever-pressing schedule, while the second (or "yoked") member of the pair earned its rewards on a progressive interval schedule. The progressive ratio schedule produced a high rate of responding for several hours, whereas the progressive interval schedule produced steady, slow rates of responding. Pyridostigmine was administered, orally, to all monkeys in doses that reduce whole blood cholinesterase levels 30% to 54% for one to three weeks at a time. The results indicate that the ability of the primates to maintain a high rate of behavior over an extended period of time (two to three hours) is affected by the initial administration of chronic pyridostigmine, while the ability to maintain a lower rate of response is not. Furthermore, repeated sets of chronic pyridostigmine administration are without affect on sustained high rates of responding.

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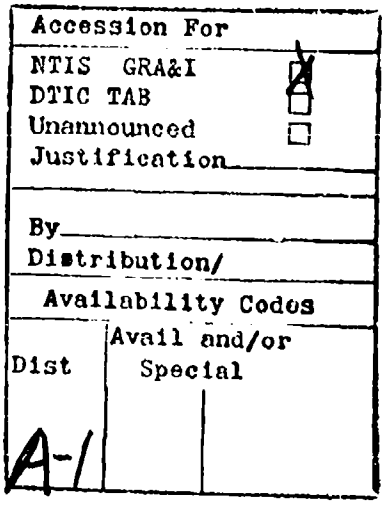
PREFACE

This work was accomplished under 875AA Pharmacology and Efficacy of Prophylactic and Therapeutic Compounds. The ICD Protocol number is 105-8-00-A-035 and the work is documented in ICD notebooks # 21-80, 38-80, and 05-82.



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INTRODUCTION

Pyridostigmine is a relatively long-acting, reversible carbamate-type cholinesterase inhibitor. This drug has been proposed for use in the field as a medical pretreatment when nerve agents are a possible agent threat. This type of drug prophylaxis is based on the concept of protecting a percentage of the acetylcholinesterase from phosphorylation and inactivation. To be effective, protection must occur before exposure to organophosphorus nerve agents. Protection of the enzyme renders it functionally inoperative since it can no longer hydrolyze acetylcholine while in its inhibited state. The physiological and behavioral consequences of inactivating different percentages of the pool of acetylcholinesterase are under study by many laboratories and countries at the present time. As a consequence of this inhibition, changes can be expected to occur at the neuromuscular junction.

Myasthenia gravis is a neuromuscular disease characterized by weakness and marked fatigability of skeletal muscle (Drachman, 1978). Studies involving these patients have shown that neostigmine improves both voluntary muscular activity and muscular response to tetanic stimulation. An equal dose of neostigmine in non-myasthenics, however, leads to a reduced muscular response to tetanic stimulation, which is accompanied by fasciculations, local weakness, and recurrent action potentials following a single stimulus (Grob, 1963).

Even in myasthenic patients, characteristics of the disease, such as increased weakness, decreased availability of acetylcholine receptors, reduced miniature endplate potential amplitudes, and damaged post-synaptic folds,can occur when excessive amounts of the anticholinesterase drug are prescribed (Drachman, 1978).

Given the neuromuscular effects of the carbamate class of anticholinesterase compounds, an appropriate test of their deleterious effects would be one that assesses muscular weakness or fatigability within the range of acetylcholinesterase inhibition that provides significant protection against multiple LD50 nerve agent challenges. The progressive ratio (PR) schedule of reinforcement is a method of studying relatively large numbers of responses over long-session times. On a PR schedule, reinforcement follows a fixed number of responses. This defines the response requirement. During the experimental trial period, the number of responses required to earn a reinforcement increases by a predetermined increment after each reinforcement. The response requirement continues to increase until the subject ceases to respond for a defined period of time or fails to complete the required number of responses within a given time limit. The response requirement that the subject fails to complete is termed the "breaking point." Hodos (1961) was the first investigator to report that the breaking point dependent variable is a sensitive measure of experimental manipulation. His original work revealed the sensitivity of progressive ration schedules to changes in the subject's deprivational level. Further research has extended the sensitivity of this baseline to include the increment increase in response requirement (Hodos & Kalman, 1963), duration of electrical brain stimulation (Hodos, 1965), changes in barometric pressure (Thomas, 1974), and the effects of drugs (Thompson, 1972). In the present experiment, the progressive ratio schedule was used to generate sustained levels of lever pressing. Chronic pyridostigmine was administered orally for periods up to four weeks and its effects were assessed through an analysis of the breaking point data.

METHODS

Subjects - Four experimentally naive, adult male cynomolgus monkeys (Macaca fascicularis), weighing between 4 to 6 kg, were used. The subjects were individually housed in stainless steel cages for primates (60 cm wide, 68 cm deep, 76 cm high) with continuous access to water. They were transported from the home cage to the experimental chamber individually by means of a transfer cage.

Apparatus - The experimental chamber was a modified home cage enclosed in a large sound-attenuated chamber located in an experimental room that was flooded with masking noise. One wall of the cage was replaced with a customized aluminum intelligence panel. The intelligence panel had three primate response levers mounted 28 cm above the grid floor. A pellet dispenser delivered a 1 gm pellet into a well that was centered below the response levers. The programming of experimental contingencies and the recording of responses were accomplished by a SCAT Behavioral Control System (Grason-Stadler Co.) and cumulative recorders (Gerbrands Co.) located in nearby rooms.

Procedure - Two pairs of monkeys were studied. One monkey of each pair worked on a progressive ratio 10 (PR10) schedule of food reinforcement. This schedule requires an increasing number of lever presses for each successive 1 gm food pellet reinforcer. The requirement increases by 10 each time. Thus, the subject had to complete 10 lever presses for the first reinforcer, 20 for the second, 30 for the third, etc. The value of the final completed ratio was defined as the "breaking point." In addition, a time requirement was programmed into the experiment such that the ratio had to be completed within an equivalent number of seconds (for example, a ratio of 50 had to be completed within 50 seconds, 200 within 200 seconds, and so on). A schedule that incorporates a time limit during which responses must be completed is called a "differential reinforcement of high rates of behavior," or DRH schedule. Thus the schedule in effect was a conjunctive PR10 DRH. This DRH contingency assured an overall rate of responding of not less than one response/second.

The availability of reinforcement for the other monkey of the pair was determined by the PR monkey earning his reinforcement. This procedure, called "yoking," allowed for the yoked monkey to earn a pellet when and only if the PR monkey completed the ratio requirement. The availability of reinforcement for the yoked monkey occurs when the PR monkey has earned its reinforcer. Reinforcement, however, is still contingent upon responding. A 10-second time limit for a response to occur was set. The yoked monkey had to respond within 10 seconds of the PR monkey's reinforcement in order to earn a pellet. Functionally, the schedule for these yoked monkeys was a Progressive Interval 10 with a 10-second limited hold (PI10 LH10). This schedule produced low but steady rates of response. The yoked animals were in separate but identical experimental chambers. No programmed cues signaling the availability of reinforcers were provided to either the PR or the PI monkeys. Yoking assured that an equal number of pellets were available to two sets of monkeys: one set was required to put out rapid, large, and increasing amounts of behavior for durations of one to three hours, whereas the second set was required to put out only small amounts of behavior at a slow, steady rate. Animals were tested during periods of chronic pyridostigmine administration for periods of one to four weeks. Animals were dosed on weekends but not tested behaviorally.

Baseline measurements of whole blood cholinesterase were made at least once in each set of baseline conditions as well as three days before and three days after the sets of dosing weeks. Additionally, cholinesterase measurements were made at least weekly while animals were on the drug. One to five milliliters of blood were drawn from a leg vein in the monkey's calf region (usually the saphenous), transferred to a test tube and kept on ice until enzymatic activity was assessed (Groff et al., 1976).

Drugs - Pyridostigmine Bromide was made into a solution of 10 mg/ml suitable for oral administration by Mr. Pete Zvirblis, US Army Medical Research Institute of Chemical Defense. A dose of 7 mg/kg was given orally twice per day, 12 hours apart, by injecting the appropriate volume into an orange or banana and giving it to the animal to eat. In all cases, animals were observed to immediately consume the drug-impregnated fruit.

Statistical Analysis - Breaking point and response data were analyzed separately for each Progressive Ratio and Progressive Interval on a per monkey basis with a BMDP program (7D Analysis of Variance) and with a Newman-Kuels post-hoc testing procedure. The significance level was set a $p < .05$.

RESULTS

Pyridostigmine Effects on Progressive Ratio Behavior - Analysis of the averaged breaking point data for Monkey W (Figure 1 & Table 1) shows that, in the first two drug runs, breaking points dropped significantly lower than in all baseline conditions and than in the subsequent three drug runs. On the first drug run, breaking points dropped after the first day and remained at approximately half their predrug value for the three weeks Monkey W was dosed (Figure 2). Breaking point values recovered on the first nondrug test day, which was the third day after drug administration ceased, and remained stable for four weeks. Breaking point values recovered on the first nondrug test ceased, and remained stable for four weeks. Breaking points showed a much slower decline for the second drug run but eventually decreased to 60% of the second baseline set of values (Figure 2). This reduction was significantly different from all baseline conditions but not significantly different from the first drug run. Recovery was much slower after the second drug run, with responding not recovering until the fifth test day (seventh day after terminating drug administration). The third drug run produced a nonsignificant change in breakpoints although a downward trend is apparent. The fourth and fifth administrations produced no changes.

Analysis of the mean number of responses revealed similar results (Table 2). The number of responses in drug runs one and two was significantly lower than in all other conditions. The maximum number of responses observed under the non-dosed runs reveals that a large amount of lever pressing behavior is produced through this schedule.

The averaged breaking points for Monkey M for the no drug and drug conditions are shown in Figure 1 and Table 1. Breaking points during the first drug run increased from the first baseline, although not significantly. The second chronic administration of the drug significantly decreased breaking points and responses (Table 1 and 2). The third and fourth drug runs were without significant effects. Statistically, the first and second drug runs were significantly lower than all other conditions except the first baseline set. The breaking point data for the successive no drug and drug conditions for Monkey M are shown in Figure 2.

Whole blood cholinesterase activity (expressed as percent of baseline value) is shown in the tables. Values for Monkey W vary between 56% and 61% of control (i.e., these values are 54% and 39% inhibition levels), while values for Monkey M vary between 52% and 71% of control (i.e., these values are 48% to 29% inhibition levels). There appears to be no correlation between the degree of response reduction observed and cholinesterase depression. For both monkeys, the greatest reduction in responding/ breaking points did not correspond with those periods of greatest depression in enzymatic activity.

Pyridostigmine Effects on Progressive Interval Behavior - Data for each animal in the Progressive Interval paradigm were analyzed in two fashions: 1) the number of food pellets earned and consumed versus the number available; and 2) the number of responses subjects made per reinforcer available. This second measure was adopted because the number of reinforcers varied as the experimental conditions changed for the PR monkeys and thus the opportunity for the PI monkeys to respond changed. Both monkeys earned and consumed all the food pellets made available to them from their PR counterparts except on six occasions. Monkey S missed one pellet twice while Monkey P missed one pellet three times and two pellets once. The missed pellets occurred in the early portions of the session when, due to the short ratios and rapid responding by the PR monkeys, pellets were available with less than 10-second separations. The averaged number of responses per reinforcer differed considerably for the two PI monkeys (Table 3). Monkey S (yoked to Monkey W) averaged 11.5 responses per reinforcer for the no drug conditions and 5.4 for the drug conditions. Monkey P (yoked to Monkey M), however, averaged 72.5 for the no drug conditions and 101.3 for the drug conditions. The difference between the drug and no drug conditions was not significant for either animal.

Chronic pyridostigmine reduced cholinesterase activity in Monkey P to an average of 56% with a range of 47% to 73%, and in Monkey S to a average of 43% with a range 25% to 50%.

DISCUSSION

The results of this study present preliminary evidence for the following:

1. high amounts of work out-put can be reduced by the initial administrations of chronic pyridostigmine, and

2. repeated sets of chronic pyridostigmine administration are without effect on sustained work outputs.

The results indicate that the behavioral effects of chronic pyridostigmine administration need to be assessed in areas where large amounts of work are required since yoked animals working at a much reduced total work load showed no drug effects. Monkey P's response level was much higher than that of the other PI monkey (S) but still considerably less than its yoked PR monkey (M) (Tables 2 & 3). The observed drug effects are probably not related to the motivational effects of food reinforcement, nor to any gastric discomfort presumably caused by this drug. A decrease in the reinforcing efficacy of food would have resulted in decreased responding in the PR group and decreased food intake in the PI group.

The observed drug effects do not seem to be related to whole blood cholinesterase inhibition. The relationship of the behavioral effects to red blood cell cholinesterase, plasma cholinesterase, or specific regions of the central nervous system's cholinesterase inhibition remain unresolved.

Importantly, it is not the high rates of behavior but rather the ability to maintain these high rates over an extended period of time that is affected by pyridostigmine administration. Analysis of the cumulative records revealed that the progressive ratio animals responded as fast as when drugged as when not drugged. Behavioral performance was typical of ratio responding at all times (rates of two to three per second) and the animals' responses never dropped below a rate of one response/second. What was

observed, however, was that the ratio animals ceased to respond after completing fewer ratio increments. Ratio monkeys worked for two to three hours under baseline conditions while working less than one hour during the first two drug administration series.

The implications of these data if verified through future experimental manipulations may have great import for the use of chronic pretreatment with a reversible inhibitor of cholinesterase like pyridostigmine. The use of such drugs and regimens may alter a soldier's ability to perform successfully in a sustained or continuous operations scenario.

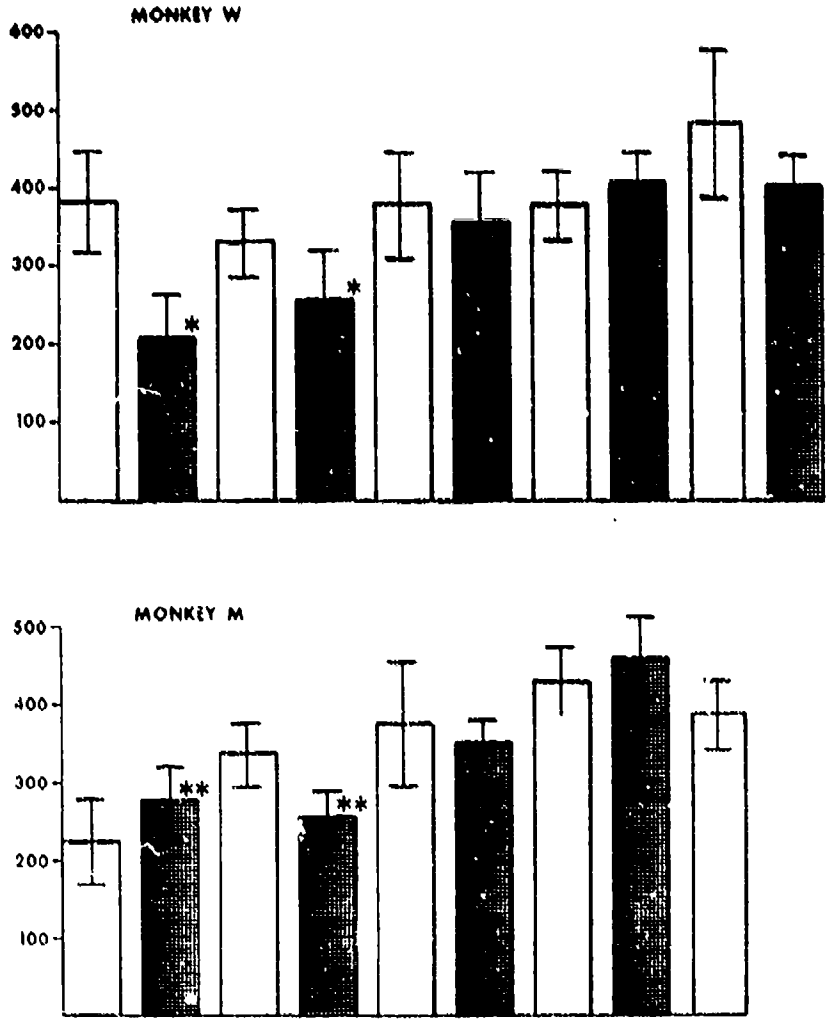

Figure 1

Progressive Ratio breaking points (ordinate) for two monkeys over no drug (open bars) and drug (hatched bars) conditions. Bars indicate averaged breaking points plus and minus one standard deviation. Asterisks indicate significant decreases in breaking points ($p < .05$) from no drug condition.

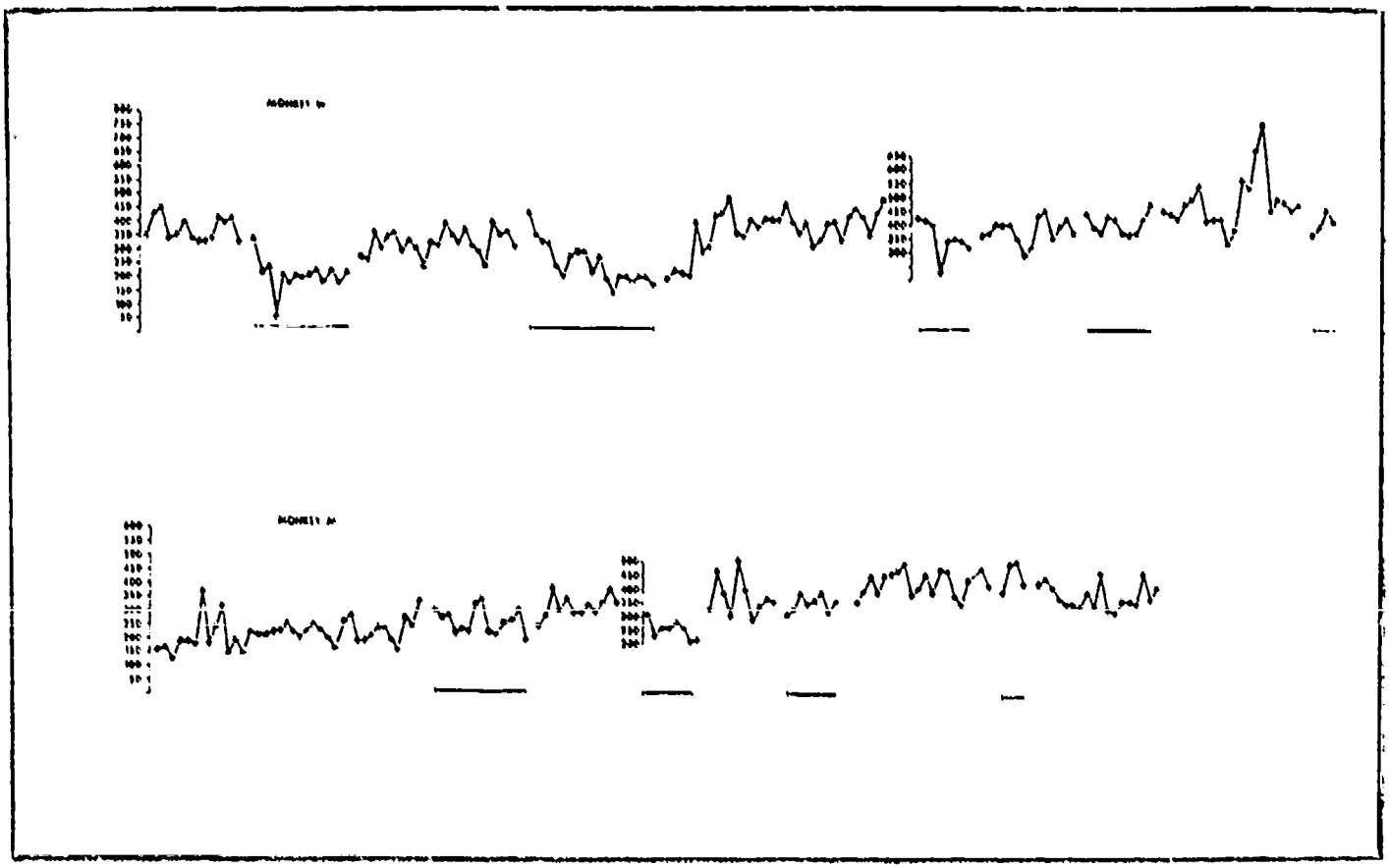

Figure 2

Session by session (abscissa) breaking points (ordinate) for two monkeys. Bars along abscissa indicate drug sessions.

TABLE 1: Mean breaking points and whole blood cholinesterase activity for two monkeys under a Progressive Ratio 10 schedule of food reinforcement: Drug vs No Drug Conditions.

Monkey W					Cholinesterase[1] Activity
Condition	Means	SD	MAX	MIN	(% control)
ND	387	64	530	320	
D	209*	58	340	60	50%
ND	330	45	410	240	
D	256*	74	440	150	61%
ND	380	79	500	200	
D	354	65	430	230	60%
ND	379	43	450	280	
D	404	37	470	360	59%
ND	482	97	760	330	
D	403	38	450	360	46%

Monkey M					Cholinesterase Activity
Condition	Means	SD	MAX	MIN	(% control)
ND	225	54	370	130	
D	277	46	360	210	52%
ND	335	41	400	260	
D	254*	33	310	210	71%
ND	377	69	510	290	
D	351	30	390	310	59%
ND	430	44	500	350	
D	460	54	510	400	54%
ND	389	46	470	310	

*$p < .05$

[1] Whole blood cholinesterase activity

TABLE 2: Mean number of responses and whole blood cholinesterase activity for two monkeys under a Progressive Ratio 10 schedule of food reinforcement: Drug vs No Drug Conditions.

Monkey W					
Condition	Means	SD	MAX	MIN	Cholinesterase[1] Activity (% control)
ND	7524	2587	13,780	5130	
D	2243*	1161	5,610	159	50%
ND	5386	1435	8,200	2821	
D	3453*	2113	9,460	1050	61%
ND	7325	2725	12,251	1900	
D	6264	2141	9,030	2534	60%
ND	7051	1566	9,700	4060	
D	8036	1505	10,810	6303	59%
ND	11818	5326	28,856	5280	
D	7975	1511	9,907	6306	46%

Monkey M					
Condition	Means	SD	MAX	MIN	Cholinesterase Activity (% control)
ND	2592	1329	6,877	789	
D	3823	1321	6,301	2115	52%
ND	5542	1330	7,800	3250	
D	3140*	833	4,650	2100	71%
ND	7133	2746	12,750	4060	
D	6054	1015	7,410	4650	59%
ND	8869	2624	12,250	630	
D	10458	2420	12,751	7800	54%
ND	7589	1685	10,810	5280	

*$p < .05$

[1] Whole blood cholinesterase activity

TABLE 3: Progressive interval responses and responses per reinforcer for two monkeys.

		Responses	Responses per Reinforcer
Monkey S	ND	316 ± 190	11.8 ± 30
	D	194 ± 90	5.4 ± 3
Monkey P	ND	2621 ± 1249	72.5 ± 35
	D	3114 ± 1179	101.3 ± 34

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